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Original Research Article

Therapeutic Potential of Stink Bug *Coridius nepalenseis* (Westwood) (Hemiptera: Dinidoridae): An *In Vitro* Study

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Abstract: Many ethnic communities in the Himalayan belt including northeast India and Bhutan consume the stinkbug *Coridius nepalensis*. In addition, some other species of *Coridius* are used as food or medicine in some other parts of the world. However, *C. nepalensis* was mainly focussed as food insect in previous works. Therefore, to evaluate its medicinal property, present work was designed to analyse the antioxidant, antiglycation and antiamyloid potential of the insect using aqueous extract (CN. Aq. Ex.). Antioxidant activity was studied using ABTS radical scavenging, DPPH radical scavenging, NO scavenging, reducing power and metal chelating power assays. Similarly, Congo red binding assay was used to study antiamyloid potential. Glucose-bovine serum albumin (BSA-glucose) assay and inhibition of fructosamine production were used to study antiglycation potential. Total polyphenol content was also estimated, as certain polyphenols are involved in many of these biochemical activities. CN. Aq. Ex. showed 33.73%, 65.16% and 80.36% scavenging activity for ABTS, DPPH and NO radical respectively. The extract also exhibited 86.10% fructosamine inhibition and 95% β-amyloid aggregation inhibition demonstrating significant antiglycation andantiamyloid potential. The total polyphenol content in CN. Aq. Ex. was found to be 45.19 ± 2.79 µg gallic acid Equivalents (GA equiv.)/mg. Present results showed that the aqueous extract of *C. nepalesis* possesses significant antioxidant activity in terms of reducing power and NO scavenging. In addition, it also exhibited significant antiglycation and antiamyloid activity.

Key words: Antiamyloid, antioxidant, antiglycation, *Coridius nepalenseis,* polyphenol

Introduction

A number of age-associated chronic diseases are in link with metabolic disorders. These include functional disruption of certain biomolecules caused by free radicals, reactive oxygen species, glycation reactions, abnormal protein aggregation etc. Oxidative metabolism, immune system and environmental pollution are known to produce a number of free radicals and reactive oxygen species. Catalases, vitamins and various antioxidants in the living organisms transform these highly reactive elements to non-reactive forms to eliminate their effect. However, the level of these reactive molecules sometimes exceeds the scavenging capacity of the body's endogenous system. As a consequence, these highly reactive molecules may result in development of tumour, atherosclerosis, cardiovascular disorders, diabetes, cirrhosis, neurodegenerative diseases, AIDS, premature ageing and inflammatory diseases (Eisenberg and Jucker 2012; Adrover *et al.*, 2014; Phaniendra

et al., 2015; Liguori *et al.,* 2018; Kruk *et al*., 2019; Moldogazieva *et al*., 2019).

Likewise, glycation is found to associate with hyperglycaemia and abnormal protein aggregation. Glycation is non-enzymatic reaction where reducing sugars binds with protein or lipid. Spontaneous glycation can result in formation of advanced glycation end products (Ulrich 2001; Adrover *et al*., 2014). These products are also considered as one of the factors responsible for aging and the development or worsening of many degenerative diseases, such as diabetes mellitus, atherosclerosis, chronic kidney disease and Alzheimer's disease (Goldin *et al*., 2006; Vistoli *et al*., 2013).

Amyloids are extracellular, insoluble protein fibril deposits resulted from abnormal protein aggregation. Such amyloids are associated with more than 50 human diseases, known as amyloidosis. These may contribute to cause some neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob disease, motor neuron diseases, the large group of polyglutamine disorders, including Huntington's disease, as well as diseases of peripheral tissue like familial amyloid polyneuropathy (Pulawski *et al*., 2012; Chiti and Dobson 2017; Benson *et al*., 2018).

Recent studies suggest certain plant and animal derived molecules to lower oxidative stress, amyloids and advanced glycation end products by their antioxidant potential (Auwera *et al*., 2005; Pham-Huy *et al*., 2008; Hanson *et al*., 2013; Liguori *et al*., 2018; Gill *et al*., 2019). Therefore, the present study aims to evaluate the antioxidant, antiglycation and antiamyloid activity of stinkbug *Coridius* (=*Aspongopus*) *nepalensis* (Westwood 1837) (Hemiptera: Dinidoridae) *in vitro*. Certain communities in Himalayan region including inhabitants of northeast India and Bhutan consume these insects (Chakravorty *et al*., 2011; Shantibala *et al*., 2012; Gogoi 2015; Rinchen 2016; Pongener *et al*., 2019). Additionally, some other species of Coridius are known to be use as food or medicine in some other parts of the world (Van Huis *et al*., 2013; Liu *et al*., 2019). *C. nepalensis* are most popularly known as 'Tari' or 'Gandhipuk' in certain local dialect in Arunachal Pradesh and adjoining areas (Gogoi *et al*., 2017) These are very often sold in market for the purpose of consumption. It has been observed that these insects are collected mainly from dry riverbed during winter season or on the way to their winter destination. Choice of these insects as a source of food in northeast India has been noted as early as beginning of 20th century. It is consumed either in roasted form or making paste of pounded uncooked adult and rice (Distant, 1906; Maxwell-Lefroy, 1909). However, the chemical analysis on this insect was mainly focussed on its nutrient contents (Chakravorty *et al*., 2011). Therefore, to evaluate its medicinal property, the present work was designed to analyse the antioxidant, antiglycation and antiamyloid potential of the insect using aqueous extract.

Materials and methods

Collection of insect sample and preparation of aqueous extract

Adult *C. nepalensis* (Fig. 1) (n= 20, both males and females in equal number) were collected from local market (Arunachal Pradesh) during winter season (October, 2018-March, 2019). They were identified as per the accessible description of Distant (1906). Collected individuals were surface cleaned using phosphate buffer saline (PBS) of pH 7.2 and stored in refrigerator (-20°C) until used to avoid breakdown of the active biomolecules. As these insects are with hard chitin, cryogenic grinding method was used to grind them. For this,

Fig. 1 *Coridius nepalensis* in its natural habitat of dry stony riverbed

the insects were ground using chilled mortar and pestle in presence of liquid nitrogen to prepare a fine paste. The paste was dissolved in 1× PBS at 1:5 ratio and centrifuged at 7,000 rpm, for 30 minutes at 4°C to separate lipid (upper layer), aqueous extract (middle layer) and impure chitin (lower layer). The aqueous extract was carefully collected and stored at -80°C overnight. It was then lyophilised and the powder obtained was used for antioxidant assays. Hereafter, for convenience, this aqueous extract will be mentioned as "CN Aq. Ex. L-ascorbic acid and gallic acid standards were purchased from Hi-media (India). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ABTS [2,22 - azino-nis (3-ethylbenzthiazoline-6-sulfuric acid)] and other chemicals were procured from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water was used in all the experiments.

In vitro antioxidant activity

(1) ABTS radical scavenging assay

ABTS [2,22 - azino-nis (3-ethylbenzthiazoline-6-sulfuric acid)] radical cation decolouration assay was done to measure radical scavenging activity, as described by Re *et al.* (1999) and Mukherjee *et al.* (2011). Ascorbic acid was used as reference standard. In this assay, ABTS is converted to its radical cation by addition of sodium persulfate. Concentration of CN Aq. Ex. within the range of 1-5 mg/ml were used for this purpose. Radical scavenging activity of the extract was assessed spectrophotometrically. Absorbance was recorded at wavelength 475 nm. Ability of the extract to scavenge ABTS radical was expressed as EC_{50} (µg/ml). The inhibitory activity was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 = absorbance of the control,

A_1 = absorbance of CN Aq. Ex.

The extract concentration providing 50% inhibition (EC_{50}) was obtained by plotting inhibition percentage versus extract concentration.

(2) DPPH radical scavenging assay

To determine the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, CN Aq. Ex. was reconstituted to 1-5 mg/ml concentrations by dissolving in phosphate buffer (pH 7.4) (Leaves and Leaves, 2014). Then, 100 µl of this solution with varying concentrations of CN Aq. Ex. was added to 96 well microtiter plates and 100 µl of a 0.2 mM DPPH solution was added to it. The solution was mixed well and incubated in dark at room temperature for 20 minutes. After incubation, the absorbance was measured at 515 nm (Eppendorf ELIZA Reader). Ascorbic acid was used as positive control. The antioxidant activity was calculated as:

$$\text{DPPH inhibitory activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 = Absorbance of positive control group,

A_1 = Absorbance in presence of CN Aq. Ex.

(3) Reducing antioxidant power (FRAP) assay

The reducing power of the aqueous extract was based on the method of Hu *et al.* (2012). 1% potassium ferric cyanide, 10% TCA, 0.1% ferric chloride, ascorbic acid (100 to 500 µg/ml), CN Aq. Ex. (1 to 5 mg/ml) were used for this assay. Absorbance of solution was recorded at 700 nm. Ascorbic acid (0.5 mM) was used standard.

(4) Nitric oxide (NO) scavenging activity

Spectrophotometric quantification of nitrite was done using Griess reagent. Nitric oxide generated from sodium nitroprusside was measured following the method of Marcocci *et al.*, (1994). For this assay, 10 µl of 5 mM sodium nitroprusside solution prepared in in PBS (pH 7.3) was added with 10 µl ascorbic acid (for standard) and 10 µl of CN Aq. Ex. (1-5 mg/ml) each in microtiter plate well. To this, 200 µl of Griess reagent was added and the mixture was kept at room temperature for 30 minutes in presence of electric light source. The resultant nitric oxide radical generated were reacted with oxygen to produce the nitrite ion, which was assayed after incubation period. Finally, absorbance of the chromophore (purple azo dye) formed was measured at 630 nm by using ELISA reader.

(5) Metal chelating ability assay

The Fe^{2+} chelation of the CN Aq. Ex. was measured following the method of Dinis *et al.*, (1994). For this, 1 to 5 mg/ml concentrations of CN Aq. Ex. were added to 100 µl of 2 mM ferrous sulphate. The reaction was initiated by the addition of

100 µl of 0.25 mM ferrozine. Mixture was mixed thoroughly and incubated at room temperature for 10 min. Ferrozine reacts with Fe^{2+} and forms a stable magenta coloured complex. For control, 100 µl of milliQ water was used in the reaction mixture. Absorbance was recorded at 517 nm and the chelating activity was calculated using the formula:

Chelating rate (%) = $[(A_0 - A_1)/A_0] \times 100$

Where, A_0 = Absorbance of control group,

A_1 = Absorbance in presence of the CN Aq. Ex.

In vitro antiglycation activity

(1) Glucose-bovine serum albumin (BSA-glucose) assay

This assay was based on the method of Brownlee *et al.* (1986). The assay evaluates the intermediate stage of protein glycation. For this, 10 mg /ml BSA and 50 mM Glucose were dissolved in phosphate buffer (100 mM, pH 7.4). 25 µl of CN Aq. Ex. was added to reaction mixture. Phosphate buffer was used as a blank. After seven days of incubation, 1% (v/v) Trichloroacetic acid (TCA) was added. The tubes were incubated for 10 min at 4 °C, centrifuged at 10,000 rpm for 10 minutes and the supernatant was discarded. The resultant pellet was dissolved in buffer at p^H 10. The level of advanced glycation end products was calculated using spectrofluorometer with excitation wavelength 370 nm and emission wavelength 440 nm.

(2) Inhibition of fructosamine production

To evaluate the fructosamine production, Thiobarbituric acid (TBA) assay was used following the protocol described by Ravan *et al.* (2016). In this assay, 100 µl of 20% Trichloroacetic acid (TCA) was added to equal volume of glycated BSA and sample was centrifuged at 10,000 rpm for 10 min. The resultant pellet was solubilized in 500 µl phosphate buffer. Then, 50 µl of this sample was added to 50 µl 40% TCA following centrifugation at 10,000 rpm for 10 min. 50 µl of obtained supernatant was mixed with 50 mM TBA, incubated in a boiling water bath for 20 min and cool down to room temperature. The absorbance was measured at the wavelength 450 nm. Inhibition of fructosamine was calculated using the formula:

Inhibitory activity (%) = $[(A_0 - A_1)/A_0] \times 100$

where, A_0 = absorbance of positive control group,

A_1 = absorbance in presence of the extract sample

In vitro inhibition of β-amyloid aggregation

β-amyloid aggregation in the samples was estimated by Congo red binding following the method of Tupe *et al.* (2015). Accordingly, 150 µl of glycated albumin was added with 30 µl of 100 mM Congo red solution in phosphate-buffered saline and incubated at room temperature for 20 min. The absorbance was measured at wavelength 530 nm.

The percentage of inhibition was measured using the equation:

Inhibition % = $[1 - (A_0 - A_1)/A_0] \times 100$

Where, A_0 = absorbance of positive control group,

A_1 = absorbance in presence of the extract

Estimation of polyphenol content

Total polyphenol content in CN. Aq. Ex. was estimated following the method of Singleton *et al.* (1999). The Folin–Ciocalteu (F C) reaction was performed using Folin–Ciocalteu reagent (1:10 W/V), 7.5% sodium carbonate, Gallic acid (1 mg/ml) at different concentrations (100 to 500 µg/ml). Gallic acid solution was used as standard and results were presented as µg of gallic acid equivalents (GAE) per mg of sample.

Statistical analysis

All the assays were done in triplicate. Results of *in vitro* antioxidant assays were statistically compared between Ascorbic acid and CN. Aq. Ex. by using t-test. For antiglycation assay, one-way analysis of variance (ANOVA) was used followed by Tukey's test (mean comparison) using statistical software GraphPad PRISM 7. Significance of the results was reported at $p < 0.05$.

Results

***In vitro* antioxidant activity**

CN Aq. Ex. showed dose dependent increase in ABTS radical, DPPH radical, NO scavenging activity, reducing power and metal chelating ability. It was observed that CN. Aq. Ex. exhibited comparatively lower ABTS radical scavenging capacity. While 100 µg/ml ascorbic acid exhibited 60.95% scavenging, 10 mg/ml CN. Aq. Ex. was showing 33.73% (Fig. 2). However, CN. Aq. Ex. showed higher DPPH radical scavenging activity compared to ABTS radical scavenging (Fig. 3). 10 mg/ml CN. Aq. Ex. showed 65.16% DPPH radical scavenging and 100 µg/

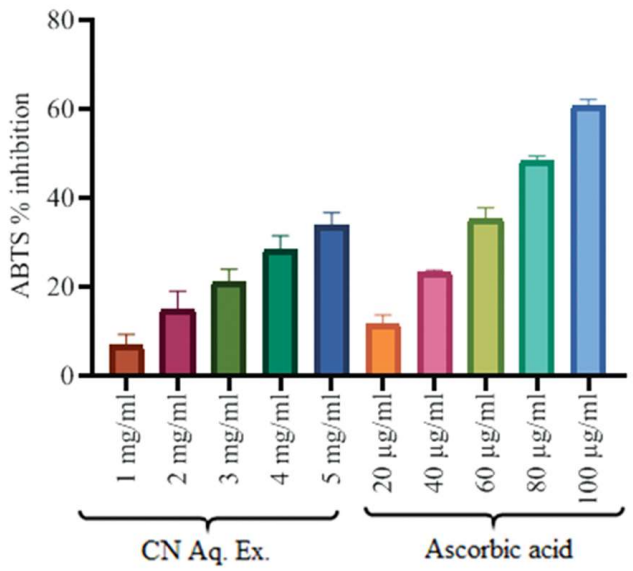


Fig. 2. ABTS radical scavenging activity of CN. Aq. Ex. (concentration of 1-5 mg/ml) compared with Ascorbic acid (concentration of 20 to 100 µg/ml), as reference standard. CN Aq. Ex. showed concentration dependent increase in % scavenging activity of ABTS radicals.

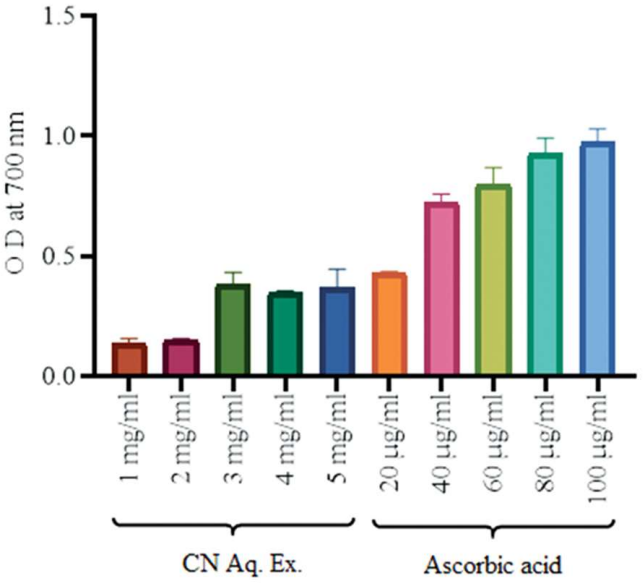


Fig. 4. Reducing power of CN. Aq. Ex. (concentration of 1-5 mg/ml) in terms of absorbance (700 nm) compared with Ascorbic acid (concentration of 20 to 100 µg/ml), as reference standard. Increase in OD indicates concentration dependent increase in reducing power activity of CN. Aq. Ex.

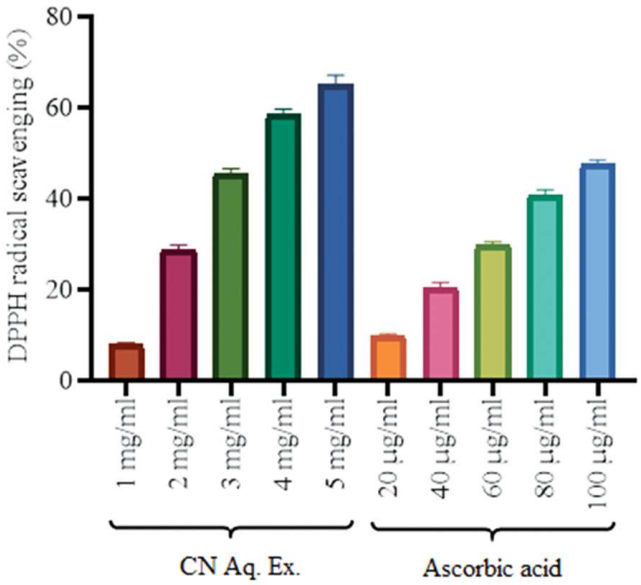


Fig. 3. DPPH radical scavenging activity of CN. Aq. Ex. (concentration of 1-5 mg/ml) compared with Ascorbic acid (concentration of 20 to 100 µg/ml), as reference standard. CN Aq. Ex. showed concentration dependent increase in % scavenging of DPPH radical.

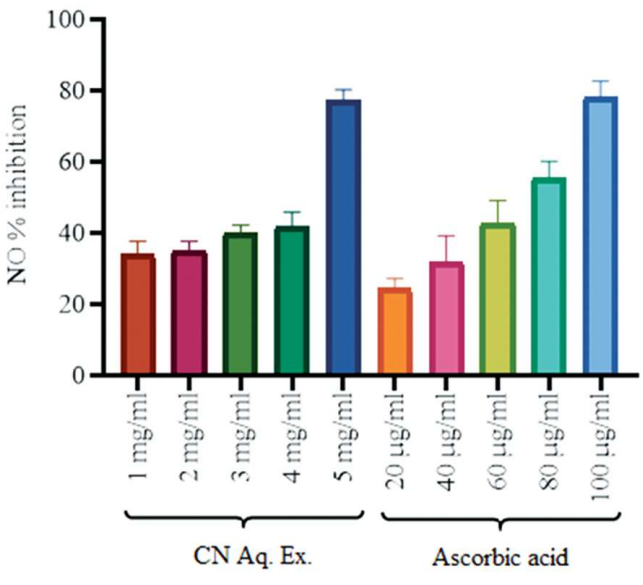


Fig. 5. NO scavenging activity of CN. Aq. Ex. (concentration of 1-5 mg/ml) compared with Ascorbic acid (concentration of 20 to 100 µg/ml), as reference standard. CN Aq. Ex. showed concentration dependent increase in % NO scavenging activity.

ml ascorbic acid showed 46.97%. Reducing power potential of 10 mg/ml CN. Aq. Ex. was found higher than that of 100 µg/ml Ascorbic acid (Fig. 4). The NO scavenging activity of CN. Aq. Ex. was found higher compared to ABTS radical and DPPH radical scavenging. 10 mg/ml CN. Aq. Ex. showed 80.36% NO scavenging and 100 µg/ml Ascorbic acid exhibited 74.34% NO scavenging (Fig. 5). Metal chelation potential is found lower for CN. Aq. Ex. (Fig. 6). The EC_{50} of reducing

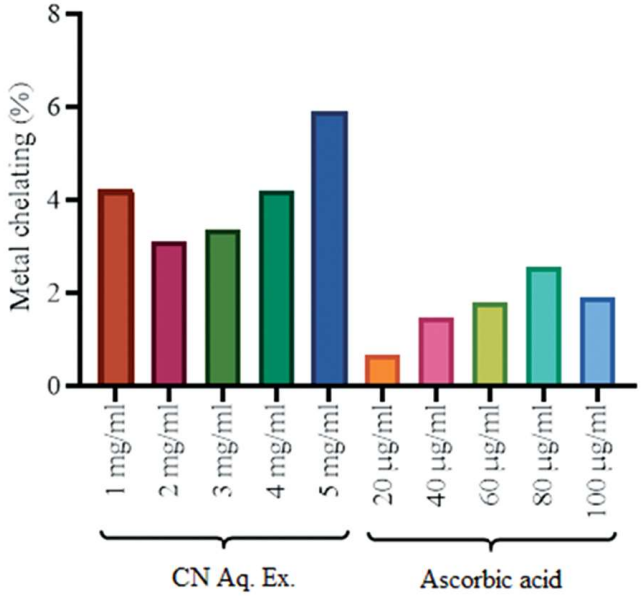
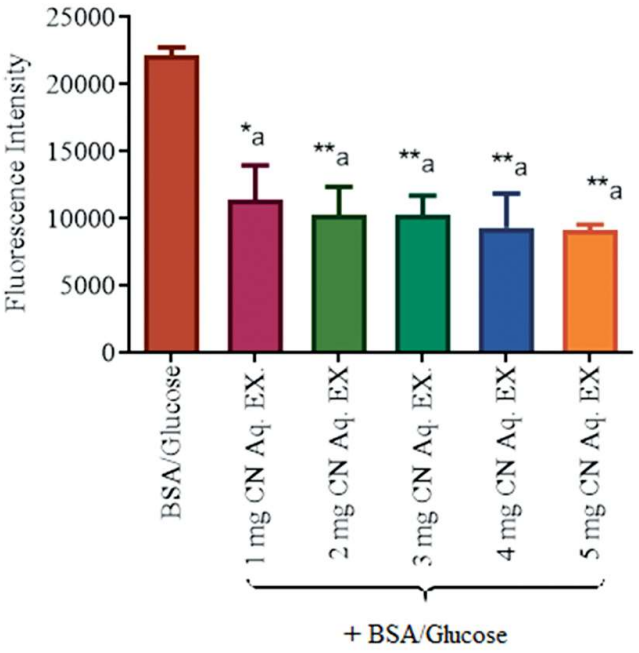


Fig. 6. Metal chelation activity of CN. Aq. Ex. (concentration of 1-5 mg/ml) compared with Ascorbic acid (concentration of 20 to 100 µg/ml), as reference standard. CN. Aq. Ex. exhibited increase in % metal chelation as per increase in its concentration used.



Data values were presented as mean ± SEM; n=3; analysed using One way ANOVA followed by Tukey's test; *=p<0.05, **= p<0.01

Fig. 7. *In vitro* antiglycation activity of CN. Aq. Ex. (concentration of 1-5 mg/ml) added with BSA-glucose complex (10 mg /ml BSA and 50 mM Glucose). Production of advanced glycation end products measured in terms of produced fluorescence intensity was inhibited in concentration dependent manner by CN. Aq. Ex.

Table 1. Antioxidant effect (EC_{50}) on ABTS radicals, DPPH radicals, FRAP assay, NO scavenging assay and metal chelating power assay of aqueous extracts of *C. nepalensis* and Ascorbic acid standard.

Name of assays	EC50 value (mg/ml)		
	Ascorbic acid	CN Aq. EX.	t-value
ABTS radical scavenging	0.823 ± 0.087	8.94 ± 0.755*	-57.9029
DPPH radical scavenging	0.219 ± 0.017	1.147 ± 0.175*	-13.1417
NO scavenging	0.362 ± 0.018	4.81 ± 0.286*	-15.4723
Reducing power	0.43 ± 0.008	0.11 ± 0.007*	32.091
Metal chelating power	0.894 ± 0.084	2.293 ± 0.03*	-26.3903

Data values were presented as mean ± SEM; n=3; analysed using Student's t-test; *=p<0.05. Degrees of freedom = 4; critical value: 2.776,
The absolute value of the calculated t exceeds the critical value, so the means are significantly different.

Table 2. *In vitro* inhibition of fructosamine and amyloid β-amyloid formation by CN. Aq. Ex.

BSA/Glucose: CN. Aq. Ex.	Antiglycation assay		Antiamyloid assay	
	Inhibition of Fructosamine (%± SE)	EC50 (mg/ ml)	Inhibition of β-amyloid (% ± SE)	EC50 (mg/ ml)
BSA/Glucose + 1 mg/ml CN. Aq. Ex	75.30 ± 13.55	0.72 ± 0.16	50.33 ± 21.61	0.775 ± 0.036
BSA/Glucose + 2 mg/ml CN. Aq. Ex.	76.20 ± 14.08		54.80 ± 26.69	
BSA/Glucose + 3 mg/ml CN. Aq. Ex.	76.87± 13.22		56.67 ± 26.82	
BSA/Glucose + 4 mg/ml CN. Aq. Ex	77.20 ± 14.72		83.90 ± 11.63	
BSA/Glucose + 5 mg/ml CN. Aq. Ex	86.10 ± 7.203		95.00 ± 1.114	

power of CN. Aq. Ex. was found the lowest. In all other assays,the EC_{50} of CN Aq. Ex. were higher compared to EC_{50} of ascorbic acid (Table 1).

In vitro inhibition of β-amyloid aggregation and antiglycation activity

CN Aq. Ex. was found to inhibit formation of advanced glycation end products, which was measured by fluorescence intensity produced in glucose-bovine serum albumin (BSA-glucose) assay. After incubation for 7 days, BSA/glucose showed increase in fluorescence intensity. CN Aq. Ex. lowers this intensity significantly ($p<0.05$) in dose dependent manner

(Fig. 7). BSA/glucose + 5 mg CN Aq. Ex. showed 42.31 % antiglycation capacity. Likewise, the CN Aq. Ex. also exhibited dose dependent inhibition to form fructosamine and β-amyloid with EC_{50} values 0.72 ± 0.16 mg/ ml and 0.775 ± 0.036 mg/ ml respectively (Table 2). 5 mg/ml CN Aq. EX exhibited 86.10% fructosamine inhibition and 95% β-amyloid aggregation inhibition showing significant antiglycation and β-amyloidactivity.

Polyphenol content

The total polyphenol content (TPC) in CN. Aq. Ex. was found to be 45.19 ± 2.79 µg Gallic Acid Equivalents (GA equiv.)/mg.

Discussion

Many substances are known to impart antioxidant behaviour. Some of the underlying mechanisms of these molecules include decomposition of peroxides into stable products, reducing the rate of peroxide formation, deactivation of catalytic activity of metal oxidants and inhibition of free-radical mediated reaction chains. Vitamins, many polyphenols, aryl amines, certain peptides, chitosan, fatty acid derivatives, terpene etc. are identified to exhibit antioxidant properties (Liu *et al*., 2012; Zieliñska *et al*., 2018; Andrade *et al*., 2019; del Hierroet *et al*., 2020; Oghenesuvwe and Paul, 2019). Polyphenols are mostly plant secondary metabolites containing more than one phenolic unit. These are moderately water-soluble compounds. Aryl amines are group of amines in which one or more hydrogen atoms of ammonia are replaced by aromatic groups. Likewise, various polyphenols, peptides, omega-3 fatty acids, steroids, alkaloid derivatives are also known to exhibit antiglycation activity and β-amyloid anti-aggregation activity (Odjakova 2012; Hjorth *et al*., 2013; Perera *et al*., 2015; Porzoor *et al*., 2015; Sharma *et al*., 2017; Yeh *et al*., 2017; Freund *et al*., 2018; Giorgetti *et al*. 2018; Phan *et al*., 2019; Piwowar *et al*., 2019; Rajasekhar *et al*., 2020).

In insects, polyphenols and their derivatives are reported from cuticles, metathoracic gland of stinkbugs etc. (Atkinson *et al*., 1973, Srinivasulu and Janaiah, 2011). In the present study, the total polyphenol content was found to be ~ 45 mgGA equiv./g. The phenolic content of the beetle, *Protaetia brevitarsis* extract was recorded as 6.32-73.53 mg GA equiv./g (Suh and Kang, 2012). *Coridius jaunas,* a close relative of *C. nepalensis*, is known to contain Iso-butenyl phenol in metathoracic gland. Similarly, it is also known to secrete N-methyldodec-6, 10-diene amine (Srinivasulu and Janaiah, 2011). The aqueous extracts from exuviae of *Periplaneta americana* was also reported to inhibit lipid autoxidation. It is known to contain antioxidant phenolics: Protocatechuic acid and 3,4-Dihydroxyphenyl Ethanol. Likewise, *Holotrichia parallela*, *Brachytrupes orientalis, Gryllodes sigillatus*,*Tenebrio molitor* and *Schistocerca gregaria* are also reported to exhibit antioxidant activity (Liu *et al*., 2012; Dutta *et al*., 2017; Zielinska *et al*., 2017; Tang *et al*., 2018).

During the present study, the EC_{50} of CN. Aq. Ex. to scavenge ABTS radical scavenging was recorded as 8.9 mg/ml and that of ascorbic acid was 0.823 mg/ml. In earlier studies, EC_{50} to scavenge ABTS radical was recorded as 0.00275 mg/ml for *G. sigillatus* protein extract, 0.0046 mg/ml for *Zophobas morio*, 0.174 and 0.149 mg/ ml for larvae and imago extracts of *P. brevitarsis* respectively (Suh and Kang, 2012, Zielinska *et al*., 2017). EC_{50} of *Patanga succincta* and *Chondracris roseapbrunner* extracts were recorded as 0.081 and 0.069 mg/ml for ABTS radical scavenging (Chatsuwan *et al*., 2018). Compared to these, CN Aq. Ex. has low ABTS radical scavenging capacity.

In this work, the EC_{50} of CN. Aq. Ex. to scavenge DPPH radicalwas recorded as 1.147 mg/ml and that of ascorbic acid as 0.219 mg/ml. The EC_{50} of protein fraction from *G. sigillatus,* extracts from *Patanga succincta* and *Chondracris roseapbrunner* against DPPH radical was recorded as 0.00691 mg/ml, 0.204 mg/ml and 0.176 mg/ml respectively (Zielinska *et al*., 2017; Chatsuwan *et al*., 2018). Thus, CN Aq. Ex. shows less effectiveness as DPPH radical scavenger.

In this study, EC_{50} of CN. Aq. Ex. to show reducing power was recorded as 0.11 mg/ml and that of ascorbic acid as 0.43 mg/ml. Reducing power of aqueous extracts was reported as 0.212 mg/ml from grasshoppers, 0.00188 mg/ml from African caterpillars, 0.00181 mg/ml cricket, 0.771 mg/ml from *G. sigillatus* and 0.364 mg/ml from *S. gregaria* protein preparation, 0.198 mg/ml from *T. molitor* peptide fraction (Zielinska *et al*., 2017).

EC_{50} of extracts from *P. succincta* and *C. roseapbrunner* were recorded as 0.022 and 0.027 mg/ml for reducing power (Chatsuwan *et al*., 2018). These data show CN Aq. Ex. to possess significant reducing power. Di Mattia (2019) reported higher reducing power from grasshoppers, African caterpillars and crickets compared to fresh orange juice.

During the present study, the EC_{50} of CN. Aq. Ex. for metal chelating power was recorded as 2.293 mg/ml and that of ascorbic acid as 0.894 mg/ml. Metal chelating ability of the peptide fraction from the *T. molitor* was reported with EC_{50} value 0.00221 mg/ml. It shows CN Aq. Ex. as less effective metal chelator. In this study, 10 mg/ml CN. Aq. Ex. showed 80.36% NO scavenging. It shows that CN Aq. EX. is also capable to scavenge substantial amount of NO. This activity along with the reducing power of CN Aq. EX may be due the presence of specific polyphenols or water-soluble peptides in the aqueous extract.

In the present study, CN. Aq. Ex. also showed significant antiglycation and antiamyloid activity showing CN Aq. Ex. as good inhibitor for of glycation product formation and amyloid aggregation. Dutta *et al.* (2017) reported increased glucose utilization in cells treated with hydro-alcoholic extracts of *Brachytrupes orientalis*. As evident from the earlier literature, these activities may also be due to the presence of polyphenols and peptides present in the aqueous extract of the insect. Liu *et al.* (2019) reported increase in antioxidant activity and decrease in apoptosis in rats after treatment with hot water extract from closely related species *C. chinensis* that is used as Chinese medicine. NO or related species are involved in regulation of several genes contributing in many biological functions including programmed cell death or apoptosis (Olson and Garbán, 2008). From the present study too, it may be concluded that CN. Aq. EX. has substantial potential as antioxidant in terms of reducing power and NO scavenging and as antiglycation and antiamyloid agent.

Conclusion

Present study shows that the stinkbug *C. nepalensis* is apotential source with antioxidant efficiency in terms of reducing power and NO scavenging. In addition, it has substantial potential as antiglycation and antiamyloid agent. Further studies to find out the bioactive ingredients for the antioxidant, antiglycation and antiamyloid activities will assist to justify strongly the health benefits of these insects in these aspects.

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Declaration of conflict of interest statement

The authors declare that there is no conflict of interest.

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